PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [4]:
A61K 39/395, 39/44, 45/02
A61K 47/00, 37/02, 49/00

A1

(11) International Publication Number: WO 89/ 06544

(43) International Publication Date: 27 July 1989 (27.07.89)

(21) International Application Number: PCT/US89/00123

(22) International Filing Date: 12 January 1989 (12.01.89)

(31) Priority Application Number: 144,541

(32) Priority Date: 15 January 1988 (15.01.88)

(33) Priority Country: US

(71) Applicant: CENTOCOR, INC. [US/US]; 244 Great Valley Parkway, Malvern, PA 19355 (US).

(72) Inventors: KLUG, Thomas, L. ; 208 Brecknock Terrace East, West Chester, PA 19380 (US). BAINE, Yaela ; 216 Edgehill Road, Merion, PA 19066 (US). MILLER, Richard, W. ; 211 Lexington Drive, Phoenixville, PA 19460 (US). LeDONNE, Norman ; 1415 Price Lane, Downingtown, PA 19335 (US).

(74) Agents: DeCONTI, Giulio, A., Jr. et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: HETEROLIGATING ANTIBODIES AND THERAPEUTIC USES THEREOF

(57) Abstract

This invention discloses a method of utilizing and augmenting a patient's own endogenously-produced bioactive compounds through the judicious use of specially designed multifunctional antibodies. In particular, the invention describes the use of heteroligating antibodies produced by polydomas in which one antigen binding site is reactive with a target and the other site is capable of initiating, or promoting the use of, at least one endogenous bioactive compound directed against the target. In another embodiment of this invention, heteroligating antibodies are described that bind a bioactive compound such as lymphokine or a metabolite and a target ligand such as a cancer cell. Specifically, heteroantibodies are described binding interferon-gamma and tumor cells. Other antibodies bind both tissue plasminogen activator and platelets.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	ML	Mali
AU	Australia	GA	Gabon	MR	Mauritania
BB	Barbados	GB	United Kingdom	MW	Malawi
BE	Belgium	HU	Hungary	NL	Netherlands
BG	Bulgaria	IT	Italy	NO	Norway
BJ	Benin	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SN	Senegal
CH	Switzerland	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
DE	Germany, Federal Republic of	LU	Luxembourg	TG	Togo
DK	Denmark	MC	Monaco	US	United States of America
FI	Finland	MG	Madagascar		

HETEROLIGATING ANTIBODIES AND THERAPEUTIC USES THEREOF

Background

The past decade has seen tremendous advances in the use of bioactive agents such as enzymes, hormones, or lymphokines (i.e. interferon-alpha, interferon-gamma, interleukin-2) for treatment of human disease. For reviews see Oldham. R. K. Inteferon 6: 127-143, (1985); Oldham, R. K., Cancer Treat Rep. 68, 221-232, (1984). See also Guarino, A. M. Methods in Cancer Res. 20:91-170, 1979 (summarizing pharmacological studies of anticancer drugs).

The increase in such experimental drug therapies is largely the result of concomitant advances in immunology and recombinant DNA technology. In particular, progress in immunology was spurred by the seminal work of Kohler and Milstein, Nature 256:495 (1975). They demonstrated that a process of somatic cell fusing between a lymphocyte and a myeloma cell, could yield immortal "hybridomas" which grow in culture and produce a specific antibody. Such antibodies are termed "monoclonal" herein and in the art.

Since that time, a new generation of antibodies has been developed with novel properties, useful for immunotherapeutic and immunodiagnostic applications. For example chimeric antibodies consisting of variable and heavy immunoglobulin regions from different species have been synthesized. In addition

it has been shown that light immunoglobulin chains from different myeloma sources can form specific heterologous associations. Peabody, D. S. et al. Biochem 19:2827 (1980). In vitro construction of antibody fragments with binding affinity for two different ligands has also been accomplished. Raso, V., Cancer Res. 41:2073 (1981).

Further modifications of the basic Kohler-Milstein method have produced individual bifunctional antibodies which are capable of binding two different antigens. The fusion of two hybridoma cell lines, each parent producing a monoclonal Ab specific for one of two antigens, yields a "quadroma". Fusion of a hybridoma and a lymphocyte yields a "trioma". Such combinations are called, "polydomas" herein and in the art. See, for example U.S. Patent No. 4,474,893 (1984); PCT Patent No. WO 83/03679 (1983) which are incorporated by reference. The uses of monoclonal antibodies, chimerics, and the multifunctional antibodies of polydomas are manifold.

These antibodies have found utility in tumor therapy (Raso and Giffin, Cancer Res. 41:2073, 1981), immunodiagnostics (Litman et al. Anal. Biochem 106:223, 1980), and immunohistology (Suresh, M. R. et al., PNAS 83:7989-7993 (1986). In most uses, recombinant monoclonal antibodies are covalently attached to a dye, drug or tracer compound. Polydomas are particularly useful in this regard. For example, a polydoma can be produced containing one binding site specific for the Ricinus communis

toxin, and the other site specific for tumor cells. A polydoma can be synthesized containing a tissue-specific antigen in combination with a monoclonal antibody to a fluorescent probe.

There are, however, drawbacks to the use of both monoclonal antibodies and bioactive compounds in immunotherapy and immunodiagnostics. Use of various lymphokines and monoclonal antibodies to which is bound a plant or bacterial toxin, radio-label, or a dye cytotoxin, have risks associated with injection into a patient. There may be generation of antibody against an injected immunogenic agent, resulting in an anti-idiotypic response in humans and a loss of immunogenic activity. See Talle, M. A. et al. (EPO No. 863,087,854). Furthermore, use of recombinant drug products is limited by the expense of treatment with large doses of exogenously produced materials (i.e., $19,000 for interleukin-2 treatment for one cancer patient). Moreover the process of drug development itself generally takes from 2 to 10 years and may involve the expenditure of tens of millions of dollars to bring a drug from concept to commercial market.

It would be useful to devise a therapeutic modality that overcomes the limitations described herein. Since nearly all diseases or pathological conditions arise because of inadequate or inappropriate bodily responses to the disease or pathological agent, a procedure utilizing and augmenting the host's own endogenously produced bioactive compounds should increase the efficacy of

therapy and make such therapy safer and far less expensive.

The present invention discloses a novel approach to the use of polydomas and bioactive compounds in therapy and diagnostics. By using polydomas in which one antigen binding site is reactive with a target and the other site capable of initiating, directing, or promoting use of at least one endogenous bioactive material, this invention discloses a method of augmenting or focussing the organism's normal response to the target by altering the local concentration(s) of the host's existing biomolecules. As will be described below, there are many diseases and pathological situations in which these multifunctional antibodies, free of toxins, dye, or radiolabels, may find clinical utility.

Summary of the Invention

The present invention provides a therapeutic procedure employing a multifunctional antibody specifically reactive with a ligand and at least one endogenous biological response modifier. The antibody serves to concentrate endogenous biological modifiers at or near the target ligand to produce a therapeutic effect. The invention provides a therapeutic method which comprises administering a pharmaceutically effective amount of a multifunctional antibody specifically reactive with both a target ligand and an endogenous biological response modifier.

Brief Description of the Drawings

Figure 1 schematically illustrates four mechanisms by which tumor cells can be killed by targeted heteroligating monoclonal antibodies.

Figure 2 shows results of the chromatographic isolation of 17-1A x B140 quadroma.

Figure 2A illustrates isolation using protein-A sepharose chromatography.

Figure 2B illustrates isolation using hydrophobic interaction chromatography (HIC).

Figure 3 shows an SDS:polyacrylamide gel of the 8B4 quadroma illustrating that 8B4 contains a 1:1 ratio of 17-1A and B140 heavy Ig chains.

Figure 4 shows an isoelectric focusing gel of 8B4 illustrating that the isoelectric point of 8B4 is the average of its parental antibodies.

Detailed Description

The invention provides a therapeutic method for concentrating naturally occurring bioactive compounds at or near a specific site or sites within a patient. As used herein, the expressions "bioactive" compound, "biological response modifier" or "endogenous biological modifier" (EBM), are used interchangeably and refer to agents produced by the patient that alter biological responses for the benefit of the patient. A partial list of endogenous biological modifiers suitable for use in this invention includes the lymphokines, such as B-cell factor, interferons and interleukins; enzymes such as tissue plasminogen activator, hormones,

immunoglobins, proteins, cofactors, metabolites, antigenic determinants, salts and ions. In preferred embodiments of this invention, the endogenous biological response modifier is a lymphokine or enzyme such as inteferon-gamma or tissue plasminogen activator (TPA), respectively.

The invention emcompasses a method of increasing concentration of these bioactive materials at a specific target site or ligand. This "target site" will be located within the body and is an antigen or one or more epitopes on the antigen. The term "antigen" is well known in the art and refers to a biological structure or "epitope", usually a protein or carbohydrate, which stimulates within the body the production of an antibody reactive with the antigen. A partial list of pre-selected target sites suitable in this invention includes normal cells such as T-cells, B-cells, endocrine, nerve cells; cancer cells, benign disease cells, vascular occlusions including fibrin and platelets; disease processes such as plaques, amyloid, and arthritic joints; parasites, hormones, bacteria, viruses, immunoglobulins, toxins and/or metabolites. Preferably, the pre-selected target site or ligand is a cancer cell or a vascular occlusion.

The therapeutic method of this invention involves the administration of multifunctional or "heteroligating" antibodies. As used herein, the term "heteroligating antibody" refers to a monoclonal antibody which contains at least two different antigen binding sites on the same molecule.

The heteroligating monoclonal antibodies (hereafter HLMAb) of the present invention are useful for binding to, and concentrating these above-mentioned endogenous bioactive molecules at the situs of target sites or ligands. The method of the present invention comprises administering a pharmaceutically effective amount of a HLMAb specifically binding to both a pre-selected target site and a bioactive material. It is thought that the HLMAb brings the target ligand in close proximity to bioactive components of the host's metabolic and homeostatic system in such a way that the host's endogenous bioactive compounds will respond to, or induce a clinically desirable response to, the target.

In other words, the present invention is a method of augmenting and/or focussing the patients normal response to the target ligand/antigen by altering the local concentration(s) of the host's endogenous biological response modifiers. It is unimportant whether the HLMAb will bind to the bioactive molecules first and subsequently to target ligands, or vice versa. The mode of action of the invention is defined operationally as either concentrating the endogenous bioactive materials at the target site and/or coating the surface of a target site with HLMAb in order to present an array of receptors for circulating bioactive agents. The invention accomplishes this by virtue of the ability of the HLMAb to bind to two or more agents simultaneously, thus bringing two or more different cell types or two or more different bioactive materials

into close proximity. Appropriate dosages of heteroligating antibodies described herein will vary according to the therapeutic effect desired.

Methods of producing HLMAb's are known in the art. In general, the HLMAb of the present invention would be formed by joining two (or more) monoclonal antibodies, at least one of which specifically binds to a naturally occuring bioactive molecule and/or to a surface antigen on an endogenous target cell that influences the biologic response of the host to a target ligand. The other part of the HLMAb may consist of a MAb which binds pre-selected target antigen, for example, to a tumor-associated antigen (TAA), a foreign antigen, or a normal cellular antigen or cell product (see attached Table I for list of possible targets and endogenous biological modifiers).

The joining of MAbs to form the HLMAb may be achieved by: 1) fusion of two (or more) parental MAb producing cells (hybridomas) into a single cell which will produce the HLMAb as a natural cell product; or 2) by fusion of a MAb producing hybridoma with a lymphocyte producing an antibody having specific binding affinity to another desired antigen. The hybrid hybridoma may be called a "quadroma" or a "polydoma" if the hybrid arises from the fusion of two hybridomas, or two or more hybridomas, respectively. The fusion product of a hybridoma and antibody producing lymphocyte is called a "trioma". One method for making triomas is disclosed in U.S. Patent 4,474,893, the relevant

disclosure of which is incorporated herein by reference. See also Lanzavecchia, A. and D. Scheidegger, Eur. J. Immunol.

There are also a variety of methods to prepare heteroligating polyclonal antibodies or HLMAbs by chemical recombinations after isolation and purification of antibody or antibody fragments. Parham, P., Human Immunol., 12:213-222 (1985).

There are many diseases and pathological situations in which the embodiments of this invention may find clincal utility. Some of these include: neoplasia (cancer); cardiovascular disease; autoimmune disease; viral, bacterial, and parasitic infections; neurologic disorders, endocrine and metabolic disorders; diseases of the blood and blood-forming organs; disease of the respiratory system; disease of the skin and subcutaneous tissue; disease of the musculoskeletal and connective tissue; congenital abnormalties; injury and poisoning.

In one embodiment of this invention, heteroligating antibodies are coadministered with exogenous naturally occurring bioactive agents such as IL-2 or interferon-gamma. In this situation, these are coadministered as an antigen-antibody complex in which the bioactive compound is already bound to the heteroligating antibody, the antibody serving to protect the bound molecule from in vivo degradation until it reaches its target. The heteroligating antibody can also be introduced over a considerable period of time to bind the greatest

amount of antibody to the tumor. The target cells then, in effect, carry receptors for the biological response modifiers and can be selectively vulnerable to a brief period of treatment with low levels of the same exogenously administered materials. This invention can also be used to more effectively eliminate, select, or remove tumor cells in vitro. An example of this is in the removal of cancerous cells from autologous bone marrow cells in vitro prior to returning them into irradiation-treated cancer patients.

In a preferred embodiment of the present invention, a heteroligating antibody with bi-specificity to both a target antigen and endogenous human interferon-gamma (IFN-gamma) can be injected into a patient with a tumor, and this can localize at the tumor site by binding to the tumor surface antigen or epitope. The anti-IFN-gamma portion of the quadroma then binds to and concentrates endogenous IFN-gamma on the tumor cells. The IFN-gamma then mediates killing of the tumor cell by at least three separate independent mechanisms; 1) direct cytotoxicity of IFN-gamma; 2) antibody-independent, monocyte-mediated cytotoxicity following activation by IFN-gamma; and 3) monocyte-independent, lymphoid-dependent cytotoxicity following IFN-gamma induction of monokines IL-1 and IL-2 to activate LAK and T-cells. Depending on the nature of the quadroma, a fourth mechanism, ADCC (Antibody Dependent Cellular Cytotoxicity) mediated

by monocytes following antibody binding to Fc receptor on the monocyte, can be involved.

The invention, its operation and its effects in concentrating endogenous IFN-gamma are illustrated in Figure 1. In the first mechanism, IFN-gamma released from the heteroligating MAb, or MAb-bound IFN-gamma pinocytosed by the tumor cell is directly cytotoxic to the cell. In the second mechanism, released or bound IFN-gamma activates nearby monocytes and these then non-specifically lyse tumor cells upon contact. In the third mechanism, monocytes are activated as before, but the lymphokines secreted by the activated monocytes (macrophages) amplify the lytic response through activation of adjacent lymphocytes. A fourth mechanism can be invoked if the heteroligating MAb binds specifically to the monocyte's Fc receptor and the bound monocytes are capable of a cytolytic ADCC response.

The present invention is not limited to full chain HLMAb's but includes those made from $(Fab')_2$ or $(FAb)_2$ fragments. Moreover, this invention is not limited to targeting malignant tumor cells, but may also be used to target any cell population, pathological organisms, or pathological molecule either in vivo or in vitro (See Table I).

TABLE I

A Partial List of Endogenous Biological Modifiers (EBMs) and Targets for Heteroligating Monoclonal Antibodies

EBMs	Targets
A. Lymphokines . B-cell factors . Interferons . IL-2	A. Cancer Cells
B. Monokines . TNF . IL-1	B. Normal Cells . T-Cells . B-Cells . Endocrine, Nerve
C. T-Cell Populations . Cytotoxic Cells . Activated T-Cells	C. Benign Disease Cells
D. Macrophages	D. Vascular Occlusions . Fibrin . Platelets
E. NK Cells	E. Disease Processes . amyloid . plaques . arthritic joints
F. Enzymes	F. Parasites
G. Hormones	G. Bacteria
I. "Contact" Proteins	H. Viruses
J. Cofactors, metabolites	I. Natural Toxins
K. "Antigens"	J. Immunoglobulins
L. Salts, Ions	K. Hormones
	L. Metabolites

An example for this application is in the delivery of macrophages to gram-negative bacteria with a polydoma composed of anti-lipopolysaccharide and anti-macrophage. Another possibility is treatment of rheumatoid arthritis with a polydoma both to a rheumatoid factor and interferon-gamma. A further example is an HLMAb reagent for use as a thrombolytic and fibrinolytic agent comprising an anti-platelet and an anti-TPA HLMAb.

The present invention increases the efficacy of therapy and makes it more economical. This is especially true in embodiments of this invention that use exogenous bioactive agents. In situations where the bioactive agent is bound to the HLMAb prior to administration, less must be injected since they are already targeted to the site.

The invention will now be illustrated by the following examples.

Example I: Preparation and Purification of Heteroligating Antibody

Monoclonal antibodies (MAb) produced by standard Kohler-Milstein immunization and hybridoma method were identified. Tumor selective MAbs were developed for colorectal, breast, and ovarian carcinomas. Antibodies to interferon-gamma, interleukin-2, tumor necrosis factor (TNF), and T-cells were also developed. All antibodies were of murine origin and were of the immunoglobulin IgG class.

Several of these hybridomas were made drug resistant or drug selectable by DNA transfection

with bacterial plasmid vectors using protoplast fusion techniques. See Knight, D.E. and M.C Scrutton, Biochem. J. 234:497, 1986; and Potter, H. et al. Proc. Natl. Acad. Sci. USA 81:7161, 1984. Bacterial DNA plasmid vectors pSV2-neo (ATCC #37149) and pSV2-gpt (ATCC #37199) were used to introduce the aminoglycoside 3- phosphotransferase (aph) and xanthine-guanine phosphoribosyltransferase (gpt) genes, respectively. The hybridomas secreting MAbs against tumor antigens were transfected with DNA to produce G418 resistance (aph+) and the hybridomas secreting MAbs to the biological response modifiers were transfected to render them resistant to mycophenolic acid (gpt+). Transfectants were cultured in increasing doses of the respective drug until they continued to grow at dose levels of drug that were at least 2-3X the dose that was absolutely lethal to all nontransfected cells (aph-, gpt-). Positive selection for successful hybridoma fusions is obtained by culturing fused hybridomas in the presence of both drugs. A true fusion of hybridomas will be doubly drug selectable (aph+, gpt+).

Several cell-cell fusions have been done with one of the hybridomas secreting MAb (17-1A, IgG2a) against a colorectal cancer antigen and one of the hybridomas secreting MAb against human interferon-gamma (B140; IgG1). Fusions were cultured and cloned in the presence of both G418 and mycophenolic acid as described above. Viable fusion-produced cell lines that grew on the double selection media were then tested for heterobifunctional activity.

This assay used an immunoaffinity purified 17-1A tumor antigen on a solid phase and radiolabelled IFN-gamma as tracer. Binding of the radiolabelled interferon tracer indicated the presence of hetero-
05 functional activity i.e. an antibody with binding sites to both 17-1A antigen and IFN-gamma.

Two heteroligating clones, 8B4 and 1B10 were selected for further recloning. Ascites and hybridoma supernatant were made for both 8B4 and
10 1B10. These two cells lines were recloned 6 times before a stable clone was eventually found. These were then expanded for use in the production of antibody from supernatants and ascites. Purification protocols for heterobifunctional antibodies
15 were developed using conventional chromatographic techniques for isolating and separating immunoglobulins including protein-A affinity, ion-exchange, and hydrophobic interaction chromatography.

20 Immunoglobulins secreted by clone 8B4 have been characterized. Clone 8B4 secretes both 17-1A and B140 parental MAbs as well as the heterobifunctional antibody. The immunoglobulins have been isolated and purified by protein-A chromatography, followed
25 by separation by hydrophobic interaction chromatography (HIC) (Figure 2A and 2B, respectively). Results indicate that 80% of the antibody produced by clone 8B4 was heteroligating. Ascites yielded as much as 1 mg/ml of the heterobifunctional 17-1A X
30 B140. The behavior of the 8B4 quadroma on SDS:PAGE gels (Figure 3), and isoelectric focussing (IEF)

gels (Figure 4) gave further evidence that 8B4 is a 1:1 classical combination of the two parental antibodies. SDS:PAGE gel assays (Figure 3) demonstrate that 8B4 contains a 1:1 ratio of both 17-1A and B140 heavy immunoglobulin chains; the IEF shows that the isoelectric point of the 8B4 is, as expected, exactly the average of the two parental antibodies (Figure 4). We have also done experiments to determine the relative affinity of the heterobifunctional for 17-1A antigen and IFN-gamma. The avidity of the IFN-gamma moiety of the 8B4 heteroantibody is largely unchanged, but the polydoma's capacity to bind to tumor 17-1A antigen is reduced by a factor of 3-4 fold. This may be due to the fact that binding of 8B4 to antigen is monovalent, that of 17-1A bivalent. There is probably no difference in intrinsic affinity between these, but the multivalent binding of the 17-1A antibody translates into greater enthalpic contribution to the free energy of association and hence greater binding at equivalent antibody concentrations.

Example 2 Targetting of IFN-Gamma to Tumor Cells Via 8B4 Heteroantibody

This Example illustrates the in vitro targeting of IFN-gamma to human colon carcinoma cells and suggests that one mechanism whereby tumor cells are killed is by antibody dependent cellular cytotoxicity (Mechanism 4; Figure 1).

Human colon HT29 (ATCC No. HTB38) at a concentration of 10^4 cells/well were cultured and labeled

with ^{125}I-uracil deoxyribose (UdR) in the presence of fluorouracil deoxyribose (FUdR) for about 24 hours. Cells were washed, then co-cultured in the presence or absence of human peripheral monocytes for about 72 hours. Monocyte concentrations were 8 or $15x10^4$ cells/well. Carcinoma and monocytes were cultured in the presence of recombinant IFN-gamma (rIFN-gamma) and either HLMAb 8B4 or an equimolar mixture of 17-1A plus B140 parental antibodies. All antibodies contained negligible amounts of endotoxin, a potent activator of macrophage (1 Endotoxin unit of bacterial lipopolysaccharide/mg antibody).

Tumor cells were allowed to lyse and the radioactivity (as ^{125}I-UdR) liberated from 100 ul of supernatant was counted. The percent cytotoxicity was calculated as the fraction of the total number of counts released from cells into the supernatant x 100 (Table 2).

TABLE 2

Effect of HLMAb 8B4 and B140+17-1A Mixture on IFN-GAMMA-Induced Monocyte Cytotoxicity

rIFN-gamma (U/ml)	HLMAb/MAb (ug/ml)	Cytoxicity (% ^{125}I-UdR Release)		
		Without Monocytes	Monocyte:Tumor Cell Ratio 8:1	15:1
0	None	0.47	4.3	5.3
	8B4(10)	-1.06	6.01(39.7)[a]	10.61(100)[a]
	B140(5)+17-1A(5)	-0.58	4.77(1.09)	8.66(63)
0.5	None	-1.23	5.42	7.07
	8B4(10)	-0.53	6.60(21.7)	11.84(67.4)
	B140(5)+17-1A(5)	-0.88	7.19(32.6)	8.31(17.6)
5	None	-0.77	8.60	13.08
	8B4(10)	-1.41	6.07(-29.4)	11.84(-9.5)
	B140(5)+17-1A(5)	-0.17	7.19(-16.3)	8.84(-32.4)

[a] Numbers in parenthesis refer to percentage change in cytotoxicity compared to antibody-free control

Addition of HLMAb 8B4 to tumor cells in the presence of IFN-gamma and monocytes showed a small, but consistent cytotoxic effect over controls with either no antibody or with a mixture of the parental MAbs. This is particularly true at higher effector:target ratios. This was the case in the presence of low levels of IFN-gamma (0 and 0.5 Units/ml). Actually IFN-gamma levels may have been higher since monocytes themselves secrete IFN-gamma. Moreover, present results may actually underestimate the ability of HLMAb 8B4 to potentiate local tumoricidal effects by targeting IFN-gamma since MAb 17-1A by itself is more effective in binding macrophage Fc receptors than is HLMAb 8B4.

The present invention discloses the generation of a 17-1A x B140 (anti-tumor x anti-interferon gamma) quadroma which is useful in therapeutic procedures since it can be used to concentrate endogenous interferon-gamma at a specific location within the body, i.e., a colorectal tumor. More generally, multifunctional antibodies of the type described herein, can be valuable as therapeutic reagents in cancer, cardiovascular disease, infectious disease, inflammatory disease, autoimmune disease and others without the need for expensive, time-consuming and possibly toxic, courses of treatment with therapeutic compounds derived from recombinant DNA technology.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiment of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A therapeutic method for concentrating naturally occurring bioactive compounds at specific sites within a patient, comprising administering a heteroligating antibody reactive with at least one such bioactive compound and reactive with at least one pre-selected target ligand.

2. A method for concentrating endogenous biological response modifiers at the site of a target ligand within the body, comprising administering an amount of antibody sufficient to bind with at least one biological response modifier and bind with at least said target ligand.

3. A method for concentrating exogenous biological response modifiers at the site of a target ligand within the body, comprising administering a multifunctional antibody capable of binding at least one biological response modifier and the target ligand in conjunction with an effective amount of biological response modifier.

4. A method of Claim 2, wherein the biological response modifier is selected from the group consisting of lymphokines, monokines, T-cell populations, macrophages, NK cells, enzymes, hormones, immunoglobulins, cofactors, metabolites, salts, ions and antigenic determinants.

5. A method of Claim 4, wherein the lymphokines are selected from the group consisting of B-cell factors, interferons, and interleukin-2; the monokines are selected from the group consisting of TNF and interleukin-one; and the T-cell populations are selected from the group consisting of cytotoxic cells and activated T-cells.

6. A method of Claim 2, wherein the target ligand is selected from the group consisting of cancer cells, normal cells, benign disease cells, vascular occlusions, disease processes, parasites, bacteria, viruses, natural toxins, immunoglobulins, hormones and metabolites.

7. A method of Claim 6, wherein the normal cells are selected from the group consisting of T-cells, B-cells, endocrine and nerve cells; the vascular occlusions are selected from the group consisting of fibrin and platelets; and the disease processes are selected from the group consisting of amyloid, plaques and arthritic joints.

8. In a method for delivering bioactive compounds to a patient, the improvement consisting of administering an amount of heteroligating antibody sufficient to specifically react with said bioactive compound and specifically react with a target ligand within the body.

9. A heteroligating monoclonal antibody specifically reactive with at least one endogenous biological response modifier and at least one preselected target ligand.

10. A heteroligating monoclonal antibody of Claim 9, wherein the biological response modifier is selected from the group consisting of lymphokines, monokines, T-cell populations, macrophages, NK cells, enzymes, hormones, immunoglobins, cofactors, metabolites, salts, ions and antigenic determinants.

11. A heteroligating monoclonal antibody of Claim 10, wherein the lymphokines are selected from the group consisting of B-cell factors, interferons, and interleukin-2; the monokines are selected from the group consisting of TNF and interleukin-one; and the T-cell populations are selected from the group consisting of cytotoxic cells and activated T-cells.

12. A heteroligating monoclonal antibody of Claim 9, wherein the target ligand is selected from the group consisting of cancer cells, normal cells, benign disease cells, vascular occlusions, disease processes, parasites, bacteria, viruses, natural toxins, immunoglobins, hormones and metabolites.

13. A heteroligating monoclonal antibody of Claim 11, wherein the normal cells are selected from the group consisting of T-cells, B-cells, endocrine and nerve cells; the vascular occlusions are selected from the group consisting of fibrin and platelets; and the disease processes are selected from the group consisting of amyloid, plaques and arthritic joints.

14. A bifunctional antibody or heteroantibody comprising:

a. at least one antigen binding region which binds specifically to a biological response modifier selected from the group consisting of lymphokines, monokines, T-cell populations, macrophages, NK cells, enzymes, hormones, immunoglobulins, co-factors, metabolites, salts, ions and antigenic determinants; and

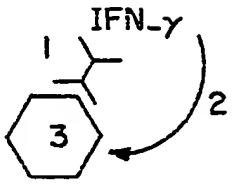
b. at least one antigen binding region specific for a target ligand selected from the group consisting of cancer cells, normal cells, benign disease cells, vascular occlusions, disease processes, parasites, bacteria, viruses, natural toxins, immunoglobulins, hormones and metabolites.

15. A heteroantibody, comprising:
    a. an antibody or antibody binding fragment specific for interferon-gamma;
    b. an antibody or antibody-binding fragment specific for tumor cells.

16. A heteroantibody, comprising:
    a. an antibody or antibody binding fragment specific for macrophage;
    b. an antibody or antibody-binding fragment specific for lipopolysaccharide.

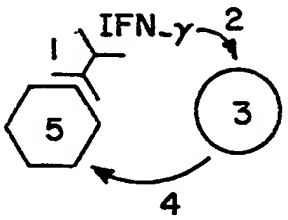
17. A heteroantibody, comprising:
    a. an antibody or antibody binding fragment specific for tissue plasminogen activator;
    b. an antibody or antibody-binding fragment specific for platelets.

MECHANISM I: DIRECT KILLING



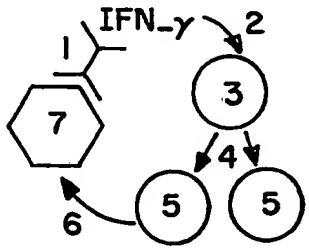
1. IFN-γ TARGETED TO TUMOR CELL
2. IFN-γ TAKEN UP BY TUMOR CELL
3. TUMOR CELL KILLED

MECHANISM 2: ACTIVATED MONOCYTE KILLING



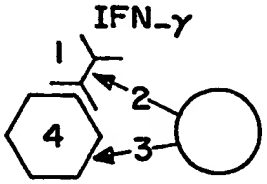
1. IFN-γ TARGETED
2. IFN-γ TO ADJACENT MONOCYTE
3. MONOCYTE ACTIVATED
4. MONOCYTES LYSES TUMOR CELL
5. TUMOR CELL KILLED

MECHANISM 3: LYMPHOKINE-ACTIVATED MONOCYTE KILLING



1. IFN-γ TARGETED
2. IFN-γ TO ADJACENT MONOCYTE
3. MONOCYTE ACTIVATED
4. MONOCYTE SECRETES LYMPHOKINES
5. MANY LYMPHOCYTES ACTIVATED
6. LYMPHOCYTES LYSE TUMOR CELL
7. TUMOR CELL KILLED

MECHANISM 4: ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY



1. HMLAb TARGETED
2. MONOCYTE BINDS TO HMLAb Fc
3. MONOCYTE LYCES TUMOR CELL
4. TUMOR CELL KILLED

FIG. 1

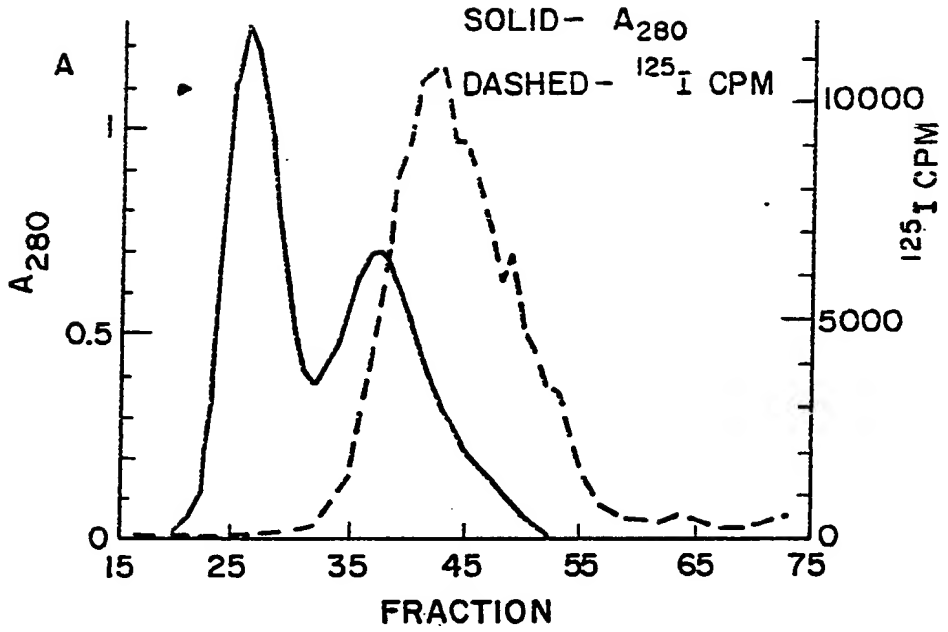

A
SOLID- A_{280}
DASHED- ^{125}I CPM
A_{280}
^{125}I CPM
1
0.5
0
10000
5000
0
15
25
35
45
55
65
75
FRACTION

FIG. 2A

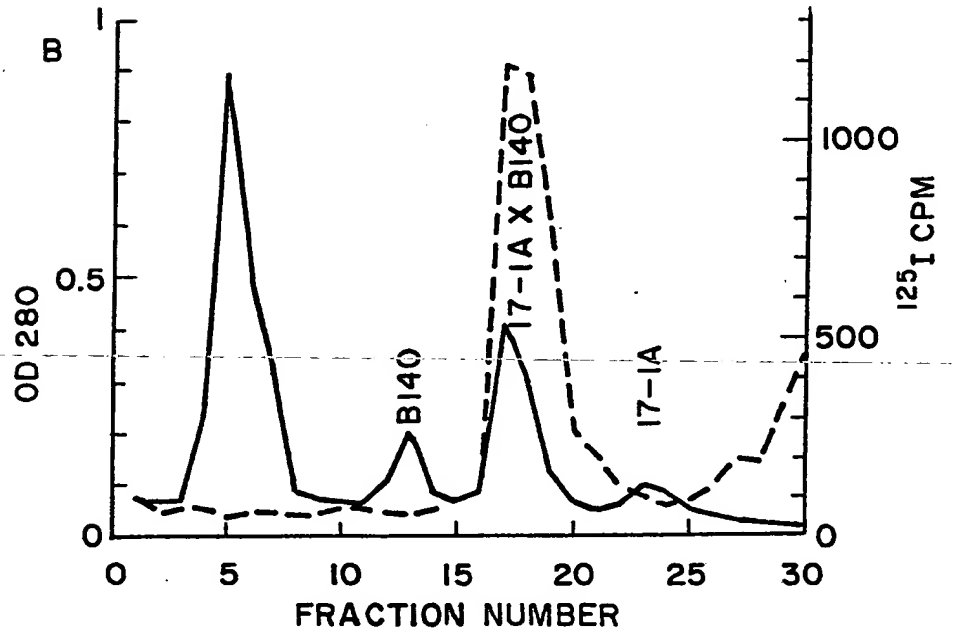

B
OD 280
^{125}I CPM
1
0.5
0
1000
500
0
B140
17-1A X B140
17-1A
0
5
10
15
20
25
30
FRACTION NUMBER

FIG. 2B

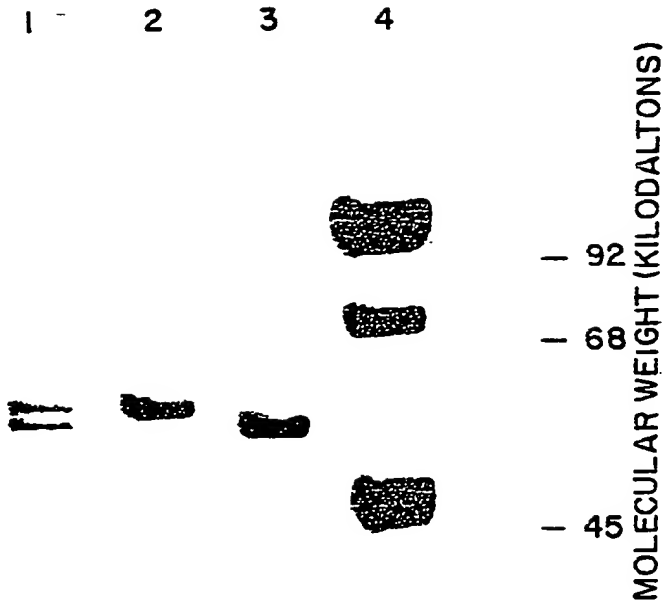

1
2
3
4
92
68
45
MOLECULAR WEIGHT (KILODALTONS)

FIG. 3

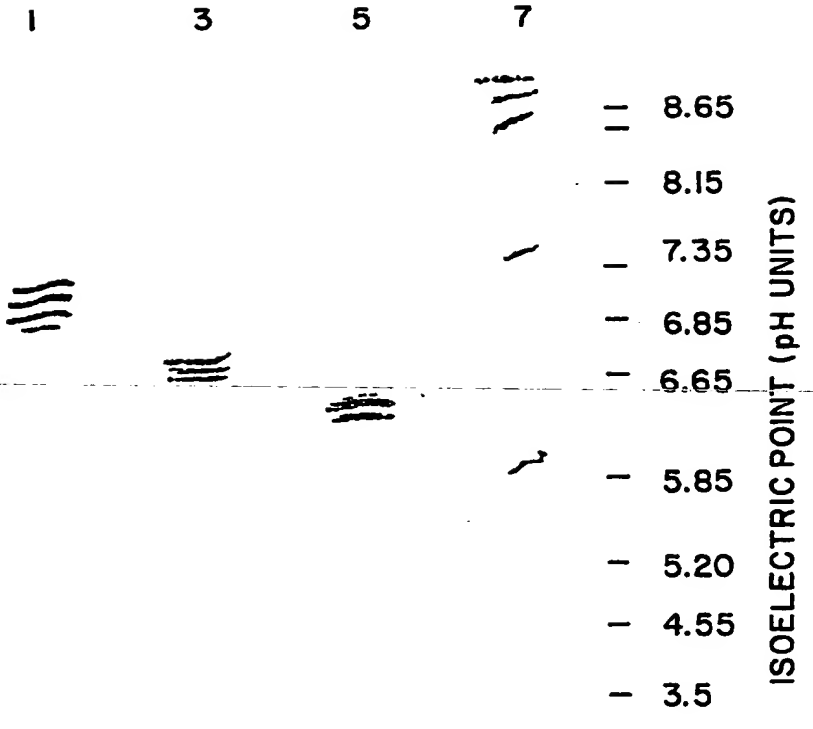

1
3
5
7
8.65
8.15
7.35
6.85
6.65
5.85
5.20
4.55
3.5
ISOELECTRIC POINT (pH UNITS)

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/00123

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC4: A 61 K 39/395, 39/44, 45/02, 47/00; A 61 K 37/02, 49/00

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC4	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	WO, A1, 85/00974 (HYBRITECH INCORPORATED) 14 March 1985, see in particular page 5, line 20 - page 6 and claims 19-25 --	9-15
X,P	JOURNAL OF INTERFERON RESEARCH, Vol. 8, 1988 SEFIK S. ALKAN ET AL.: "Enhanced Antiproliferative Action of Interferon Targeted by Bispecific Monoclonal Antibodies ", see page 25 - page 33 see in particular page 30-33 --	9-15
P,X	EP, A2, 0 294 703 (DANA-FARBER CANCER INSTITUTE, INC.) 14 December 1988, see in particular page 10 lines 33-38 and claims 31 and 42. --	9-12, 14

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th April 1989	27 APR 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	US, A, 4 613 459 (H. I. CANTOR ET AL.) 23 September 1986, see col. 5-6 --	9-14
X	WO, A1, 88/00052 (TRUSTEES OF DARMOUTH COLLEGE) 14 January 1988, see in particular pages 5, 6 and 38-42 --	9,10,12, 14
X	EP, A2, 0 241 907 (THE GENERAL HOSPITAL CORPORATION) 21 October 1987, see in particular col. 1-3 and claims	9,10,12-14
Y	--	17
X	Journal of the National Cancer Institute, Vol. 79, No. 6, December 1987 M.R. Clark et al.: "T-Cell Killing of Target Cells Induced by Hybrid Antibodies:Comparison of Two Bispecific Monoclonal Antibodies ", see page 1393 - page 1401 see in particular pages 1399-1400 --	9,10,12, 14
X	Eur. J. Immunol., Vol. 17, 1987 U.D. Staerz et al.: "Hybrid antibody-mediated lysis of virus-infected cells ", see page 571 - page 574 see page 573, right col. --	9,10,12, 14
X	The Journal of Immunology, Vol. 139, No. 5, September 1987 D.P. Snider et al.: "Targeted antigen presentation using crosslinked antibody heteroaggregates ", see page 1609 - page 1616 see in particular page 1614 --	9,10,12, 14
X	Journal of Experimental Medicine, Vol. 163, January 1986 P. Perez et al.: "Specific targeting of human peripheral blood T cells by heteroaggregates containing anti-T3 crosslinked to anti-target cell antibodies ", see page 166 - page 178 see in particular pages 174-176 --	9,10,12, 14
X	Nature, Vol. 314, April 1985 U.D. Staerz et al.: "Hybrid antibodies can target sites for attack by T cells ", see page 628 - page 631 see the whole document	9,10,12, 14

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Chemical Abstracts, volume 101, no. 13, 24 September 1984, (Columbus, Ohio, US), Hayashi Kiyotaka et al: "Analysis of cell surface molecules on human platelets with monoclonal antibodies. Identification of four platelet specific cell surface molecules". see page 477, abstract 108670m, & Nagoya Igaku 1984, 106(3), 171-9 (Japan)	17

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1-8, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv)

Method for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 89/00123

SA 26511

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 03/03/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 85/00974	14/03/85	NONE	
EP-A2- 0 294 703	14/12/88	JP-A- 64003128	06/01/89
US-A- 4 613 459	23/09/86	WO-A- 84/01504	26/04/84
		EP-A- 0122936	31/10/84
		US-A- 4720482	19/01/88
		US-A- 4722998	02/02/88
WO-A1- 88/00052	14/01/88	EP-A- 0255249	03/02/88
		AU-D- 75271/87	14/01/88
EP-A2- 0 241 907	21/10/87	WO-A- 87/06240	22/10/87

EPO FORM P079

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82